

**PCT**WORLD INTELLECTU.  
Intern

B56

WO 9604388A1

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C12N 15/62, C07K 14/54, 16/00, 19/00, A61K 38/19, 39/395</b>		A1	(11) International Publication Number: <b>WO 96/04388</b> (43) International Publication Date: 15 February 1996 (15.02.96)
(21) International Application Number: PCT/EP95/03036 (22) International Filing Date: 28 July 1995 (28.07.95) (30) Priority Data: 9415379.8 29 July 1994 (29.07.94) GB 08/468,297 6 June 1995 (06.06.95) US (71) Applicants (for all designated States except US): SMITHK- LINE BEECHAM PLC [GB/GB]; New Horizons Court, Brentford, Middlesex TW8 9EP (GB). SMITHKLINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, P.O. Box 7929, Philadelphia, PA 19103 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): BROWNE, Michael, Joseph [GB/GB]; SmithKline Beecham Pharmaceuticals, Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD (GB). MURPHY, Kay, Elizabeth [GB/GB]; SmithK- line Beecham Pharmaceuticals, Coldharbour Road, The Pin- nacles, Harlow, Essex CM19 5AD (GB). CHAPMAN, Con- rad, Gerald [GB/GB]; SmithKline Beecham Pharmaceu- ticals, Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD (GB). CLINKENBEARD, Helen, Elizabeth [US/GB]; SmithKline Beecham Pharmaceuticals, Coldhar- bour Road, The Pinnacles, Harlow, Essex CM19 5AD (GB).			YOUNG, Peter, Ronald [US/US]; SmithKline Beecham Pharmaceuticals, Research & Development, 709 Swedeland Road, King of Prussia, PA 19406 (US). SHATZMAN, Al- lan, Richard [US/US]; SmithKline Beecham Pharmaceuti- cals, Research & Development, 709 Swedeland Road, King of Prussia, PA 19406 (US). (74) Agent: WEST, Vivien; SmithKline Beecham, Corporate Intel- lectual Property, SB House, Great West Road, Brentford, Middlesex TW8 9BD (GB). (81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).  Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.
(54) Title: NOVEL COMPOUNDS			
(57) Abstract  A soluble protein having IL4 and/or IL13 antagonist or partial antagonist activity comprises an IL4 mutant or variant fused to at least one human immunoglobulin constant domain or fragment thereof.			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

## NOVEL COMPOUNDS

The present invention relates to antagonists of human interleukin 4 (IL4) and/or human interleukin 13 (IL13) for the treatment of conditions resulting from undesirable actions of IL4 and/or IL13 such as certain IgE mediated allergic diseases,

- 5 T cell mediated autoimmune conditions and inappropriate immune responses to infectious agents.

Interleukins are secreted peptide mediators of the immune response. Each of the known interleukins has many effects on the development, activation, proliferation and differentiation of cells of the immune system. IL4 has a physiological role in  
 10 such functions, but can also contribute to the pathogenesis of disease. In particular IL4 is associated with the pathway of B lymphocyte development that leads to the generation of IgE antibodies that are the hallmark of allergic diseases such as extrinsic asthma, rhinitis, allergic conjunctivitis, atopic dermatitis and anaphylaxis. IL4 can also act as a general growth and differentiation factor for T lymphocytes that  
 15 may contribute to tissue damage in certain autoimmune conditions such as insulin dependent diabetes, multiple sclerosis and rheumatoid arthritis and in graft rejection. IL4 can also suppress the generation of cell-mediated responses required for the control of infectious disease. Antagonism of the effect of IL4 on T or B lymphocytes can therefore be expected to have beneficial effects on such diseases. IL13 has been  
 20 recently identified and shares similarity in many of the biological properties of IL4 (Minty, A. *et al* (1993), *Nature* 362, 248-250) including some aspect(s) of receptor structure/function (Aversa, G. *et al* (1993), *J. Exp. Med.* 178, 2213-2218).

Human IL4 consists of a single polypeptide chain of 129 amino acids with 2 possible N-glycosylation sites and 6 cysteines involved in 3 disulphide bridges (Le, H.V. *et al.*, (1988), *J. Biol. Chem.* 263, 10817-10823). The amino acid sequence of  
 25 IL4 and the positions of these disulphide bridges are known (Carr, C. *et al.*, (1991) *Biochemistry* 30, 1515-1523).

```

                                10
30  HIS-LYS-CYS-ASP-ILE-THR-LEU-GLN-GLU-ILE-ILE-LYS-THR-LEU-ASN-
                                20                                30
    SER-LEU-THR-GLU-GLN-LYS-THR-LEU-CYS-THR-GLU-LEU-THR-VAL-THR-
                                40
35  ASP-ILE-PHE-ALA-ALA-SER-LYS-ASN-THR-THR-GLU-LYS-GLU-THR-PHE-
                                50                                60
    CYS-ARG-ALA-ALA-THR-VAL-LEU-ARG-GLN-PHE-TYR-SER-HIS-HIS-GLU-
40                                70
    LYS-ASP-THR-ARG-CYS-LEU-GLY-ALA-THR-ALA-GLN-GLN-PHE-HIS-ARG-
                                80                                90
  
```

HIS-LYS-GLN-LEU-ILE-ARG-PHE-LEU-LYS-ARG-LEU-ASP-ARG-ASN-LEU-  
 100  
 TRP-GLY-LEU-ALA-GLY-LEU-ASN-SER-CYS-PRO-VAL-LYS-GLU-ALA-ASN-  
 5  
 110 120  
 GLN-SER-THR-LEU-GLU-ASN-PHE-LEU-GLU-ARG-LEU-LYS-THR-ILE-MET-  
 129  
 10 ARG-GLU-LYS-TYR-SER-LYS-CYS-SER-SER

The disulphide bridges are between residues 3 and 127, 24 and 65, and 46 and 99. The molecular weight of IL4 varies with the extent of glycosylation from 15KDa (no glycosylation) to 60KDa or more (hyperglycosylated IL4).

15 The DNA sequence for human IL4 has also been described by Yokota, T. *et. al.*, P.N.A.S. 1986 83 5894-5898.

WO 93/10235 describes certain mutants of IL4 which are IL4 antagonists or partial antagonists.

20 EP-A-0 464 533 discloses fusion proteins comprising various portions of the constant region of immunoglobulin molecules together with another human protein or part thereof.

The present invention provides a soluble protein having IL4 and/or IL13 antagonist or partial antagonist activity, comprising an IL4 mutant or variant fused to least one human immunoglobulin constant domain or fragment thereof.

25 The term "mutant or variant" encompasses any molecule such as a truncated or other derivative of the IL4 protein which retains the ability to antagonise IL4 and/or IL13 following internal administration to a human. Such other derivatives can be prepared by the addition, deletion, substitution, or rearrangement of amino acids or by chemical modifications thereof.

30 DNA polymers which encode mutants or variants of IL4 may be prepared by site-directed mutagenesis of the cDNA which codes for IL4 by conventional methods such as those described by G. Winter *et al* in Nature 1982, 299, 756-758 or by Zoller and Smith 1982; Nucl. Acids Res., 10, 6487-6500, or deletion mutagenesis such as described by Chan and Smith in Nucl. Acids Res., 1984, 12, 2407-2419 or by G.  
 35 Winter *et al* in Biochem. Soc. Trans., 1984; 12, 224-225 or polymerase chain reaction such as described by Mikaelian and Sergeant in Nucleic Acids Research, 1992, 20, 376.

40 As used herein, "having IL4 and/or IL13 antagonist or partial antagonist activity" means that, in the assay described by Spits *et al* (J. Immunology 139, 1142 (1987)), IL4-stimulated T cell proliferation is inhibited in a dose-dependent manner.

Suitable IL4 mutants are disclosed in WO 93/10235, wherein at least one amino acid, naturally occurring in wild type IL4 at any one of positions 120 to 128

inclusive, is replaced by a different natural amino acid. In particular, the tyrosine naturally occurring at position 124 may be replaced by a different natural amino acid, such as glycine or, more preferably, aspartic acid.

The immunoglobulin may be of any subclass (IgG, IgM, IgA, IgE), but is preferably IgG, such as IgG1, IgG3 or IgG4. The said constant domain(s) or fragment thereof may be derived from the heavy or light chain or both. The invention encompasses mutations in the immunoglobulin component which eliminate undesirable properties of the native immunoglobulin, such as Fc receptor binding and/or introduce desirable properties such as stability. For example, Angal S., King D.J., Bodmer M.W., Turner A., Lawson A.D.G., Roberts G., Pedley B. and Adair R., *Molecular Immunology* vol30pp105-108, 1993, describe an IgG4 molecule where residue 241 (Kabat numbering) is altered from serine to proline. This change increases the serum half-life of the IgG4 molecule. Canfield S.M. and Morrison S.L., *Journal of Experimental Medicine* vol173pp1483-1491, describe the alteration of residue 248 (Kabat numbering) from leucine to glutamate in IgG3 and from glutamate to leucine in mouse IgG2b. Substitution of leucine for glutamate in the former decreases the affinity of the immunoglobulin molecule concerned for the Fcγ RI receptor, and substitution of glutamate for leucine in the latter increases the affinity. EP0307434 discloses various mutations including an L to E mutation at residue 248 (Kabat numbering) in IgG.

The constant domain(s) or fragment thereof is preferably the whole or a substantial part of the constant region of the heavy chain of human IgG, most preferably IgG4. In one aspect the IgG component consists of the CH2 and CH3 domains and the hinge region of IgG1 including cysteine residues contributing to inter-heavy chain disulphide bonding, for example residues 11 and 14 of the IgG1 hinge region (Frangione B. and Milstein C., *Nature* vol216pp939-941, 1967). Preferably the IgG1 component consists of amino acids corresponding to residues 1-4 and 6-15 of the hinge, 1-110 of CH2 and 1-107 of CH3 of IgG1 described by Ellison J., Berson B. and Hood L. E., *Nucleic Acids Research* vol10, pp4071-4079, 1982. Residue 5 of the hinge is changed from cysteine in the published IgG1 sequence to alanine by alteration of TGT to GCC in the nucleotide sequence. In another aspect the IgG component is derived from IgG4, comprising the CH2 and CH3 domains and the hinge region including cysteine residues contributing to inter-heavy chain disulphide bonding, for example residues 8 and 11 of the IgG4 hinge region (Pinck J.R. and Milstein C., *Nature* vol216pp941-942, 1967). Preferably the IgG4 component consists of amino acids corresponding to residues 1-12 of the hinge, 1-110 of CH2 and 1-107 of CH3 of IgG4 described by Ellison J., Buxbaum J. and Hood L., *DNA* vol1pp11-18, 1981. In one example of a suitable mutation in IgG4, residue 10

of the hinge (residue 241, Kabat numbering) is altered from serine (S) in the wild type to proline (P) and residue 5 of CH2 (residue 248, Kabat numbering) is altered from leucine (L) in the wild type to glutamate (E).

5 Fusion of the IL4 mutant or variant to the Ig constant domain or fragment is by C-terminus of one component to N-terminus of the other. Preferably the IL4 mutant or variant is fused via its C-terminus to the N-terminus of the Ig constant domain or fragment.

In a preferred aspect, the amino acid sequence of the fusion protein of the invention is represented by SEQ ID No:4, SEQ ID No:7 or SEQ ID No:10.

10 In a further aspect, the invention provides a process for preparing a compound according to the invention which process comprises expressing DNA encoding said compound in a recombinant host cell and recovering the product.

The DNA polymer comprising a nucleotide sequence that encodes the compound also forms part of the invention.

15 In a preferred aspect the DNA polymer comprises or consists of the sequence of SEQ ID No:3, SEQ ID No:6 or SEQ ID No:9.

The process of the invention may be performed by conventional recombinant techniques such as described in Maniatis *et. al.*, Molecular Cloning - A Laboratory Manual; Cold Spring Harbor, 1982 and DNA Cloning vols I, II and III (D.M. Glover ed., IRL Press Ltd).

In particular, the process may comprise the steps of:

- i) preparing a replicable expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes said compound;
- ii) transforming a host cell with said vector;
- 25 iii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said compound; and
- iv) recovering said compound.

30 The invention also provides a process for preparing the DNA polymer by the condensation of appropriate mono-, di- or oligomeric nucleotide units.

The preparation may be carried out chemically, enzymatically, or by a combination of the two methods, *in vitro* or *in vivo* as appropriate. Thus, the DNA polymer may be prepared by the enzymatic ligation of appropriate DNA fragments, by conventional methods such as those described by D. M. Roberts *et al* in  
35 Biochemistry 1985, 24, 5090-5098.

The DNA fragments may be obtained by digestion of DNA containing the required sequences of nucleotides with appropriate restriction enzymes, by chemical

synthesis, by enzymatic polymerisation on DNA or RNA templates, or by a combination of these methods.

Digestion with restriction enzymes may be performed in an appropriate buffer at a temperature of 20°-70°C, generally in a volume of 50µl or less with 0.1-10µg

5 DNA.

Enzymatic polymerisation of DNA may be carried out *in vitro* using a DNA polymerase such as DNA polymerase I (Klenow fragment) in an appropriate buffer containing the nucleoside triphosphates dATP, dCTP, dGTP and dTTP as required at a temperature of 10°-37°C, generally in a volume of 50µl or less.

10 Enzymatic ligation of DNA fragments may be carried out using a DNA ligase such as T4 DNA ligase in an appropriate buffer at a temperature of 4°C to ambient, generally in a volume of 50µl or less.

The chemical synthesis of the DNA polymer or fragments may be carried out by conventional phosphotriester, phosphite or phosphoramidite chemistry, using solid  
15 phase techniques such as those described in 'Chemical and Enzymatic Synthesis of Gene Fragments - A Laboratory Manual' (ed. H.G. Gassen and A. Lang), Verlag Chemie, Weinheim (1982), or in other scientific publications, for example M.J. Gait, H.W.D. Matthes, M. Singh, B.S. Sproat, and R.C. Titmas, Nucleic Acids Research, 1982, 10, 6243; B.S. Sproat and W. Bannwarth, Tetrahedron Letters, 1983, 24, 5771;  
20 M.D. Matteucci and M.H. Caruthers, Tetrahedron Letters, 1980, 21, 719; M.D. Matteucci and M.H. Caruthers, Journal of the American Chemical Society, 1981, 103, 3185; S.P. Adams *et al.*, Journal of the American Chemical Society, 1983, 105, 661; N.D. Sinha, J. Biernat, J. McMannus, and H. Koester, Nucleic Acids Research, 1984, 12, 4539; and H.W.D. Matthes *et al.*, EMBO Journal, 1984, 3, 801. Preferably an  
25 automated DNA synthesizer is employed.

The DNA polymer is preferably prepared by ligating two or more DNA molecules which together comprise a DNA sequence encoding the compound. A particular process in accordance with the invention comprises ligating a first DNA molecule encoding a said IL4 mutant or variant and a second DNA molecule  
30 encoding a said immunoglobulin domain or fragment thereof.

The DNA molecules may be obtained by the digestion with suitable restriction enzymes of vectors carrying the required coding sequences or by use of polymerase chain reaction technology.

The precise structure of the DNA molecules and the way in which they are  
35 obtained depends upon the structure of the desired product. The design of a suitable strategy for the construction of the DNA molecule coding for the compound is a routine matter for the skilled worker in the art.

The expression of the DNA polymer encoding the compound in a recombinant host cell may be carried out by means of a replicable expression vector capable, in the host cell, of expressing the DNA polymer. The expression vector is novel and also forms part of the invention.

5       The replicable expression vector may be prepared in accordance with the invention, by cleaving a vector compatible with the host cell to provide a linear DNA segment having an intact replicon, and combining said linear segment with one or more DNA molecules which, together with said linear segment, encode the compound, under ligating conditions.

10       The ligation of the linear segment and more than one DNA molecule may be carried out simultaneously or sequentially as desired.

Thus, the DNA polymer may be preformed or formed during the construction of the vector, as desired.

15       The choice of vector will be determined in part by the host cell, which may be prokaryotic, such as *E. coli*, or eukaryotic, such as mouse C127, mouse myeloma, chinese hamster ovary or Hela cells, fungi e.g. filamentous fungi or unicellular yeast or an insect cell such as *Drosophila*. The host cell may also be a transgenic animal. Suitable vectors include plasmids, bacteriophages, cosmids and recombinant viruses derived from, for example, baculoviruses, vaccinia or Semliki Forest virus.

20       The preparation of the replicable expression vector may be carried out conventionally with appropriate enzymes for restriction, polymerisation and ligation of the DNA, by procedures described in, for example, Maniatis *et al.*, cited above. Polymerisation and ligation may be performed as described above for the preparation of the DNA polymer. Digestion with restriction enzymes may be performed in an  
25       appropriate buffer at a temperature of 20°-70°C, generally in a volume of 50µl or less with 0.1-10µg DNA.

30       The recombinant host cell is prepared, in accordance with the invention, by transforming a host cell with a replicable expression vector of the invention under transforming conditions. Suitable transforming conditions are conventional and are described in, for example, Maniatis *et al.*, cited above, or "DNA Cloning" Vol. II, D.M. Glover ed., IRL Press Ltd, 1985.

35       The choice of transforming conditions is determined by the host cell. Thus, a bacterial host such as *E. coli* may be treated with a solution of CaCl<sub>2</sub> (Cohen *et al.*, Proc. Nat. Acad. Sci., 1973, 69, 2110) or with a solution comprising a mixture of RbCl, MnCl<sub>2</sub>, potassium acetate and glycerol, and then with 3-[N-morpholino]-propane-sulphonic acid, RbCl and glycerol. Mammalian cells in culture may be transformed by calcium co-precipitation of the vector DNA onto the cells.



The invention also extends to a host cell transformed with a replicable expression vector of the invention.

Culturing the transformed host cell under conditions permitting expression of the DNA polymer is carried out conventionally, as described in, for example, 5 Maniatis *et al* and "DNA Cloning" cited above. Thus, preferably the cell is supplied with nutrient and cultured at a temperature below 45°C.

The expression product is recovered by conventional methods according to the host cell. Thus, where the host cell is bacterial, such as *E. coli* it may be lysed physically, chemically or enzymatically and the protein product isolated from the 10 resulting lysate. If the product is to be secreted from the bacterial cell it may be recovered from the periplasmic space or the nutrient medium. Where the host cell is mammalian, the product may generally be isolated from the nutrient medium.

The DNA polymer may be assembled into vectors designed for isolation of stable transformed mammalian cell lines expressing the product; e.g. bovine 15 papillomavirus vectors or amplified vectors in chinese hamster ovary cells (DNA cloning Vol.II D.M. Glover ed. IRL Press 1985; Kaufman, R.J. *et al.*, Molecular and Cellular Biology 5, 1750-1759, 1985; Pavlakis G.N. and Hamer, D.H., Proceedings of the National Academy of Sciences (USA) 80, 397-401, 1983; Goeddel, D.V. *et al.*, European Patent Application No. 0093619, 1983).

20 Compounds of the present invention have IL4 and/or IL13 antagonist activity and are therefore of potential use in the treatment of conditions resulting from undesirable actions of IL4 and/or IL13 such as IgE mediated allergic diseases and T cell mediated autoimmune conditions or chronic microbial infection.

The invention therefore further provides a pharmaceutical composition 25 comprising a compound of the invention and a pharmaceutically acceptable carrier.

In use the compound will normally be employed in the form of a pharmaceutical composition in association with a human pharmaceutical carrier, diluent and/or excipient, although the exact form of the composition will depend on the mode of administration. The compound may, for example, be employed in the 30 form of aerosol or nebulisable solution for inhalation or sterile solutions for parenteral administration.

The dosage ranges for administration of the compounds of the present invention are those to produce the desired effect on the IL4 and/or IL13 mediated condition, for example whereby IgE antibody mediated symptoms are reduced or 35 progression of the autoimmune disease is halted or reversed. The dosage will generally vary with age, extent or severity of the medical condition and contraindications, if any. The unit dosage can vary from less than 1mg to 300mg, but

typically will be in the region of 1 to 20mg per dose, in one or more doses, such as one to six doses per day, such that the daily dosage is in the range 0.02-40mg/kg.

Compositions suitable for injection may be in the form of solutions, suspensions or emulsions, or dry powders which are dissolved or suspended in a suitable vehicle prior to use.

Fluid unit dosage forms are prepared utilising the compound and a pyrogen-free sterile vehicle. The compound, depending on the vehicle and concentration used, can be either dissolved or suspended in the vehicle. Solutions may be used for all forms of parenteral administration, and are particularly used for intravenous infection. In preparing solutions the compound can be dissolved in the vehicle, the solution being made isotonic if necessary by addition of sodium chloride and sterilised by filtration through a sterile filter using aseptic techniques before filling into suitable sterile vials or ampoules and sealing. Alternatively, if solution stability is adequate, the solution in its sealed containers may be sterilised by autoclaving. Advantageously additives such as buffering, solubilising, stabilising, preservative or bactericidal, suspending or emulsifying agents and/or local anaesthetic agents may be dissolved in the vehicle.

Dry powders which are dissolved or suspended in a suitable vehicle prior to use may be prepared by filling pre-sterilised drug substance and other ingredients into a sterile container using aseptic technique in a sterile area. Alternatively the drug and other ingredients may be dissolved in an aqueous vehicle, the solution is sterilised by filtration and distributed into suitable containers using aseptic technique in a sterile area. The product is then freeze dried and the containers are sealed aseptically.

Parenteral suspensions, suitable for intramuscular, subcutaneous or intradermal injection, are prepared in substantially the same manner, except that the sterile compound is suspended in the sterile vehicle, instead of being dissolved and sterilisation cannot be accomplished by filtration. The compound may be isolated in a sterile state or alternatively it may be sterilised after isolation, e.g. by gamma irradiation. Advantageously, a suspending agent for example polyvinylpyrrolidone is included in the composition to facilitate uniform distribution of the compound.

Compositions suitable for administration via the respiratory tract include aerosols, nebulisable solutions or microfine powders for insufflation. In the latter case, particle size of less than 50 microns, especially less than 10 microns, is preferred. Such compositions may be made up in a conventional manner and employed in conjunction with conventional administration devices.

In a further aspect there is provided a method of treating conditions resulting from undesirable actions of IL4 and/or IL13 which comprises administering to the sufferer an effective amount of a compound of the invention.

The invention further provides a compound of the invention for use as an active therapeutic substance, in particular for use in treating conditions resulting from undesirable actions of IL4 and/or IL13.

5 The invention also provides the use of a compound of the invention in the manufacture of a medicament for treating conditions resulting from undesirable actions of IL4 and/or IL13.

No unexpected toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

10 The following Examples illustrate the invention.

### **Example 1 IL4.Y124D/IgG1 fusion protein**

15 The construction of an IL4.Y124D/IgG1 chimeric cDNA, the expression of the corresponding protein in a mammalian expression system and its activity are described.

#### **1. Construction of DNA coding for fusion protein**

##### **(a) Construction of IL4.Y124D coding region**

20 A variant of the human IL4 gene, which has been described (Kruse, N, Tony, H-P and Sebal, W. EMBO Journal 11: 3237 [1992]) in which residue 124 in the protein has been mutated from tyrosine in the wild type to aspartic acid, was produced by PCR mutagenesis of the human IL4 cDNA (purchased from British Biotechnology). The IL4.Y124D cDNA was inserted into the expression vector pTR312, using the HindIII and BglII sites, (M J Browne, J E Carey, C G Chapman, A  
25 W R Tyrrell, C Entwisle, G M P Lawrence, B Reavy, I Dodd, A Esmail & J H Robinson. Journal of Biological Chemistry 263: 1599, [1988]) to form the plasmid pDB906.

30 To amplify the IL4.Y124D molecule and add convenient restriction sites at each end for subcloning, a PCR reaction was performed using 20ng of the pDB906 plasmid as the substrate. PCR primers were designed to include restriction enzyme sites, flanked by 10-15 nucleotide base pairs to "anchor" the primers at each end. The primer sequences were as follows:

35 1) 5' CGA ACC ACT GAA TTC CGC ATT GCA GAG ATA 3'  
(includes an EcoRI restriction site, GAATTC)

2) 5' CAC AAA GAT CCT TAG GTA CCG CTC GAA CAC TTT GA 3'  
(includes a KpnI restriction site, GGTACC)

Primers were used at a final concentration of 5ng/ $\mu$ l, and dNTPs were added at a final concentration of 0.2mM in a total reaction volume of 100 $\mu$ l. 31 cycles of PCR were performed. Cycles consisted of a denaturation step of 1 minute at 94°C, an annealing step of 1 minute 30 seconds at 50°C, and an elongation step of 1 minute 30 seconds at 72°C. On cycle 1 denaturation was extended to 5 minutes and on the final cycle elongation was extended to 7 minutes. 2.5 units of the Taq polymerase enzyme from Advanced Biotechnologies were used in the PCR reaction. A PCR product of 587bp was produced. This was purified using the Promega "Magic PCR cleanup" kit, and then digested with EcoRI and KpnI in react buffer 4 (all restriction enzymes were obtained from GibcoBRL.), to generate 'sticky ends'. After 4 hours 30 minutes at 37°C, the reaction was heated to 70°C for 10 minutes and then ethanol precipitated. Analysis of the resulting DNA by agarose gel electrophoresis showed the presence of three bands of approximately 570bp, 463bp and 100bp. The 570bp fragment represents the full-length IL4.Y124D variant of IL4 and was present because the digest was incomplete. The two smaller fragments were produced due to the presence of an EcoRI site within the IL4.Y124D cDNA. The 570bp band was purified by the GeneClean™ procedure, and ligated into Bluescript KS<sup>+</sup>™ which was prepared by digestion with EcoRI and KpnI followed by GeneClean™. A Bluescript KS<sup>+</sup>/IL4.Y124D recombinant was thus generated. Large amounts of this recombinant DNA were produced using the Promega "Magic Maxiprep" method. The IL4.Y124D insert was excised from the Bluescript recombinant using SmaI and KpnI. 20 $\mu$ g recombinant DNA was incubated with 25 units SmaI in react buffer 4, at 30°C overnight. 25 units of KpnI were then added to the digest, which was incubated at 37°C for 5 hours. The resulting fragment of approximately 580bp was purified by GeneClean™ to generate an IL4.Y124D/SmaI/KpnI fragment.

(b) Construction of IgG1 coding region

The COSFcLink vector (Table 1) contains human IgG1 cDNA encoding amino acids 1-4 and 6-15 of the hinge, 1-110 of CH2 and 1-108 of CH3 described by Ellison J., Berson B. and Hood L. E., Nucleic Acids Research vol10, pp4071-4079, 1982. Residue 5 of the hinge is changed from cysteine in the published IgG1 sequence to alanine by alteration of TGT to GCC in the nucleotide sequence. This was cloned from the human IgG plasma cell leukemia ARH-77 (American Type Tissue Collection), using RT-PCR and fully sequenced to confirm identity with the published sequence [patent application publication WO 92/00985]

The construction of COSFc began with a pUC18 vector containing the human IgG1 cDNA above (pUC18-Fc), which was digested with KpnI and SacII, deleting the CH1, hinge and part of CH2. The deleted region was replaced with a PCR

amplified fragment containing the hinge-CH2 region as follows. Using the following PCR primers:

5' TCG AGC TCG GTA CCG AGC CCA AAT CGG CCG ACA AAA CTC ACA  
 5 C 3'  
 and  
 5' GTA CTG CTC CTC CCG CGG CTT TGT CTT G 3'

A DNA fragment containing the hinge-CH2 region was amplified from  
 10 pUC18-Fc, digested with KpnI and SacII, gel purified and cloned into the KpnI/SacII  
 digested pUC18-Fc vector. The Cys, which occurs at position 230 (Kabat numbering;  
 Kabat et al., "Sequences of Proteins of Immunological Interest, 5th Edition, US  
 Department of Health and Human Services, NIH Publication No. 91-3242 (1991)) of  
 the IgG1 heavy chain, was altered to an Ala through a TGT to GCC substitution in  
 15 the nucleotide sequence. An altered DNA sequence in one of the PCR primers  
 introduced a unique KpnI site at the 5' end of the hinge. The resulting plasmid was  
 called pUC18Fcmmod, and the junctions and PCR amplified region were sequenced for  
 confirmation.

The entire hinge-CH2-CH3 insert in pUC18-Fcmmod was removed in a single  
 20 DNA fragment with KpnI and XbaI, gel purified, and ligated into SFcR1Cos4 cut  
 with KpnI and XbaI to create COSFc.

SFcR1Cos4 is a derivative of pST4DHFR (Deen, K, McDougal, JS, Inacker,  
 R, Folena-Wasserman, G, Arthos, J, Rosenberg, J, Maddon, PJ, Axel, R, and Sweet,  
 RW. Nature 331: 82 [1988] ) and contains the soluble Fc receptor type I (sFcR1)  
 25 inserted between the cytomegalovirus (CMV) promoter and bovine growth hormone  
 (BGH) polyadenylation regions, and also contains the dihydrofolate reductase  
 (DHFR) cDNA inserted between the  $\beta$ -globin promoter and SV40 polyadenylation  
 regions, an SV40 origin of replication, and an ampicillin resistance gene for growth in  
 bacteria. Cutting the vector with KpnI and XbaI removes the sFcR1 coding region, so  
 30 that the COSFc vector contains the hinge-CH2-CH3 region inserted between the  
 CMV promoter and BGH polyA regions.

The COSFcLink vector was made from COSFc by inserting an  
 oligonucleotide linker at the unique EcoRI site of the vector, which recreates this  
 EcoRI site, and also introduces BstEII, PstI and EcoRV cloning sites. The  
 35 oligonucleotides used were:

5' AATTCGGTTACCTGCAGATATCAAGCT 3'  
 3' GCCAATGGACGTCTATAGTTCGATTAA 5'

The junction was sequenced to confirm orientation in the vector. The size of the final vector is 6.37 kb.

5 (c) Construction of DNA coding for fusion protein.

To insert the IL4.Y124D cDNA, the COSFcLink vector was prepared by digesting with EcoRV and KpnI as follows: 5µg DNA was incubated with 15 units EcoRV in react 2 at 37°C for 5 hours, followed by ethanol precipitation. The resulting DNA was digested with KpnI in react 4 at 37°C for 3 hours, and ethanol  
10 precipitated. The IL4.Y124D/SmaI/KpnI and the COSFcLink/EcoRV/KpnI fragments were ligated together to form plasmid pDB951, which encodes the IL4.Y124D/IgG1 fusion protein. The ligation was achieved using an Amersham DNA ligation kit, product code RPN 1507, the reactions being incubated at 16°C overnight. The ligation reaction products were transformed into Promega JM109  
15 competent cells (high efficiency) and plated onto Luria Broth agar containing ampicillin at 50µg/ml. Transformants were cultured in Luria Broth (containing ampicillin at 50µg/ml) and DNA prepared using Promega "Magic Minipreps". Production of an IL4.Y124D/COSFcLink recombinant DNA was verified by restriction digests and DNA sequencing. The complete IL4.Y124D sequence and the  
20 junctions with the COSFcLink DNA were confirmed by DNA sequencing (Table 2). The coding sequence of the recombinant IL4.Y124D/IgG1 DNA is shown in Table 3 and the amino acid sequence of the fusion protein is shown in Table 4. The IL4.Y124D/COSFcLink recombinant DNA was prepared and purified using caesium chloride gradients and the DNA used to transiently transfect HeLa cells.

25

2. Expression of the fusion protein

HeLa cells were grown in MEMα medium (Gibco) with 10% foetal calf serum and 1% glutamine. For the assay,  $1 \times 10^6$  HeLa cells were seeded in 15mls RPMI-1640 medium with 10% newborn calf serum, 1% glutamine ("seeding  
30 medium"), in a 75cm<sup>2</sup> flask, four days prior to transfection. On the day prior to transfection, a further 12.5mls seeding medium was added to each flask. On the day of transfection, the medium was changed to 15mls of "transfection medium" (MEM medium with Earle's salts containing 10% newborn calf serum and 1% non essential amino acids), at time zero. At time +3 hours, 25µg of the appropriate DNA in  
35 0.125M CaCl<sub>2</sub>, 1x HBS (HEPES buffered saline) was added to the cells. At time +7 hours, the cells were subjected to a glycerol shock (15%v/v) and then left to incubate overnight in 12.5mls seeding medium containing 5mM sodium butyrate. The next day the cells were washed with PBS (Dulbecco's phosphate buffered saline) and

12.5mls "harvest medium" (RPMI-1640 with 2% of a 7.5% stock sodium bicarbonate solution) was added. After a further 24 hour incubation, the supernatants were removed, centrifuged at 1000rpm for 5 minutes to remove cell debris and stored at either 4°C or -20°C.

5

### 3. Biological Activity

For assay of supernatant for IL4 antagonist activity: using the method described in Spits et al., J. Immunology 139, 1142 (1987), human peripheral blood lymphocytes were incubated for three days with phytohaemagglutinin, a T cell mitogen, to upregulate the IL4 receptor. The resultant blast cells were then stimulated for a further three days with IL4. Proliferation was measured by the incorporation of <sup>3</sup>H thymidine.

The IL4.Y124D/IgG1 chimera inhibited <sup>3</sup>H thymidine incorporation by human peripheral blood T lymphocytes stimulated with 133pM IL4 in a dose dependent manner.

15

### Example 2

#### IL4.Y124D/IgG4 fusion protein

#### 1. Construction of DNA coding for fusion protein

PCR was performed to amplify the IL4.Y124D coding region and introduce a silent nucleotide substitution at the 3' end which creates a XhoI site. As substrate for the PCR reaction 20ng of linearised pDB951 plasmid (Example 1.1(c)) was used. The oligonucleotide primers used were as follows:

25

1) 5' CAC AAG TGC GAT ATC ACC TTA CAG GAG ATC 3'  
(includes an EcoRV restriction site, GATATC)

30

2) 5' CTC GGT ACC GCT CGA GCA CTT TGA GTC TTT 3'  
(includes a XhoI restriction site, CTCGAG).

35

A second PCR reaction was performed to amplify the hinge-CH2-CH3 fragment of the human IgG4 heavy chain. The substrate for this was a synthetic human IgG4 heavy chain cDNA, the sequence of which is described in Table 5, and is based on the Genbank sequence GB:HUMIGCD2 (Ellison J., Buxbaum J. and Hood L.E., DNA 1:11-18, 1981). Numerous silent substitutions were made to the published nucleotide sequence. The gene was assembled by combining two 0.5Kb synthetic DNA fragments. Each 0.5Kb fragment was made by annealing a series of

overlapping oligonucleotides and then filling in the gaps by PCR. The two 0.5Kb fragments were joined at the SacII site and inserted into the pCR2 vector. A 1.0Kb ApaI-BglII fragment containing the entire constant region was isolated and ligated into an expression vector, pCD, containing a humanized IL4 specific variable region.

5 This construct was used as the PCR substrate to amplify the hinge-CH2-CH3 region of IgG4.

The oligonucleotide primers used for amplification of the IgG4 hinge-CH2-CH3 region were as follows:

- 10 1) 5' GGT GGA CAA CTC GAG CGA GTC CAA ATA TGG 3'  
(includes a XhoI restriction site, CTCGAG)
- 2) 5' TTA CGT AGA TCT AGA CTA CAC TCA TTT ACC 3'  
(includes an XbaI site, TCTAGA).

15

The conditions for both PCR reactions were as described for the derivation of pDB951. Briefly, primers were used at 5ng/μl, and dNTPs at a final concentration of 0.2mM in a total reaction volume of 100μl. 2.5 Units of Taq polymerase enzyme from Advanced Biotechnologies were used and 31 cycles of PCR performed. Cycles consisted of a denaturation step of 1 minute at 94°C, an annealing step of 1 minute 30 seconds at 50°C, and an elongation step of 1 minute 30 seconds at 72°C. On cycle 1 denaturation was extended to 5 minutes and on the final cycle elongation was extended to 7 minutes.

20

PCR products of approximately 700bp (hinge-CH2-CH3 of IgG4) and 400bp (IL4.Y124D) were obtained and purified using the Promega "Magic PCR cleanup" kit. The purified PCR reactions were then digested with the following enzymes to create "sticky ends": XhoI and XbaI for IgG4 and EcoRV and XhoI for IL4.Y124D. The digests were incubated at 37°C for 3 hours and then ethanol precipitated. The resulting DNAs were analysed by gel electrophoresis and gave sizes of approximately 690bp (hinge-CH2-CH3 of IgG4) and 370bp (IL4.Y124D).

25

30

A vector was prepared into which to ligate the hinge-CH2-CH3 of IgG4 and IL4.Y124D PCR fragments by digesting pDB951 (IL4.Y124D in COSFcLink) with EcoRV and XbaI to remove most of the IL4.Y124D/IgG1 fusion molecule. The only part remaining is approximately 75bp at the 5' end of IL4, which is not present in the IL4.Y124D EcoRV/XhoI fragment produced by PCR amplification. 5μg of pDB951 DNA was digested in a total volume of 30μl using react 2 buffer (GibcoBRL). The resulting 5.8Kb DNA fragment was purified using the GeneClean<sup>TM</sup> procedure.

35



The three fragments described (IL4.Y124D EcoRV/XhoI, hinge-CH2-CH3 of IgG4 XhoI/XbaI and the 5.8Kb fragment resulting from EcoRV/XbaI digestion of pDB951) were ligated together to form plasmid pDB952, which encodes the IL4.Y124D/IgG4 fusion protein. The ligation was carried out using a DNA ligation kit from Amersham (product code RPN 1507), incubating the reactions at 16°C overnight. The ligation reaction products were transformed into Promega JM109 competent cells (high efficiency) and plated onto Luria Broth agar containing ampicillin at 50µg/ml. Transformants were cultured in Luria Broth (containing ampicillin at 50µg/ml) and DNA prepared using Promega "Magic Minipreps". Production of an IL4.Y124D/IgG4 recombinant DNA was verified by restriction digests, and the complete IL4.Y124D and hinge-CH2-CH3 IgG4 regions were verified by DNA sequencing. Table 6 describes the sequence of the coding region only of the IL4.Y124D/IgG4 fusion molecule, and Table 7 contains the amino acid sequence of the fusion protein. The IL4.Y124D/IgG4 recombinant DNA was prepared and purified using caesium chloride gradients and the DNA used to transiently transfect HeLa cells.

## 2. Expression of the fusion protein

HeLa cells were grown in MEMα medium (Gibco) with 10% foetal calf serum and 1% glutamine. For the assay,  $1 \times 10^6$  HeLa cells were seeded in 15mls RPMI-1640 medium with 10% newborn calf serum, 1% glutamine ("seeding medium"), in a 75cm<sup>2</sup> flask, four days prior to transfection. On the day prior to transfection, a further 12.5mls seeding medium was added to each flask. On the day of transfection, the medium was changed to 15mls of "transfection medium" (MEM medium with Earle's salts containing 10% newborn calf serum and 1% non essential amino acids), at time zero. At time +3 hours, 25µg of the appropriate DNA in 0.125M CaCl<sub>2</sub>, 1x HBS (HEPES buffered saline) was added to the cells. At time +7 hours, the cells were subjected to a glycerol shock (15%v/v) and then left to incubate overnight in 12.5mls seeding medium containing 5mM sodium butyrate. The next day the cells were washed with PBS (Dulbecco's phosphate buffered saline) and 12.5mls "harvest medium" (RPMI-1640 with 2% of a 7.5% stock sodium bicarbonate solution) was added. After a further 24 hour incubation, the supernatants were removed, centrifuged at 1000rpm for 5 minutes to remove cell debris and stored at either 4°C or -20°C.

## 3. Biological Activity

For assay of supernatant for IL4 antagonist activity: using the method described in Spits et al., J. Immunology 139, 1142 (1987), human peripheral blood lymphocytes were incubated for three days with phytohaemagglutinin, a T cell

mitogen, to upregulate the IL4 receptor. The resultant blast cells were then stimulated for a further three days with IL4. Proliferation was measured by the incorporation of <sup>3</sup>H thymidine.

- 5 The IL4.Y124D/IgG4 chimera inhibited <sup>3</sup>H thymidine incorporation by human peripheral blood T lymphocytes stimulated with 133pM IL4 in a dose dependent manner.

### Example 3 IL4.Y124D/IgG4 PE fusion protein

10

#### 1. Construction of DNA coding for fusion protein

PCR is performed to amplify the IL4.Y124D coding region and introduce a silent nucleotide substitution at the 3' end which creates a XhoI site as described in Example 2.

15

A second PCR reaction is performed to amplify the hinge-CH2-CH3 fragment of the human IgG4 heavy chain PE variant. In IgG4 PE, residue 10 of the hinge (residue 241, Kabat numbering) is altered from serine (S) in the wild type to proline (P) and residue 5 of CH2 (residue 248, Kabat numbering) is altered from leucine (L) in the wild type to glutamate (E). Angal S., King D.J., Bodmer M.W.,  
20 Turner A., Lawson A.D.G., Roberts G., Pedley B. and Adair R., Molecular Immunology vol30pp105-108, 1993, describe an IgG4 molecule where residue 241 (Kabat numbering) is altered from serine to proline. This change increases the serum half-life of the IgG4 molecule.

The IgG4 PE variant was created using PCR mutagenesis on the synthetic  
25 human IgG4 heavy chain cDNA described in Table 5, and was then ligated into the pCD expression vector. It was this plasmid which was used as the substrate for the PCR reaction amplifying the hinge-CH2-CH3 fragment of IgG4 PE. The sequence of the IgG4 PE variant is described in Table 8. The residues of the IgG4 nucleotide sequence which were altered to make the PE variant are as follows:  
30 referring to Table 8:

residue 322 has been altered to "C" in the PE variant from "T" in the wild type;  
residue 333 has been altered to "G" in the PE variant from "A" in the wild type; and  
35 residues 343-344 have been altered to "GA" in the PE variant from "CT" in the wild type.

Oligonucleotide primers are used for amplification of the IgG4 PE variant hinge-CH2-CH3 region as described for the derivation of pDB952.

PCR products of approximately 700bp (hinge-CH2-CH3 of IgG4 PE mutant) and 400bp (IL4.Y124D) are obtained and purified using the Promega "Magic PCR cleanup" kit. The purified PCR reactions are then digested with the following enzymes to create "sticky ends": XhoI and XbaI for IgG4 PE and EcoRV and XhoI for IL4.Y124D. The digests are incubated at 37°C for 3 hours and then ethanol precipitated. The resulting DNAs are of sizes of approximately 690bp (hinge-CH2-CH3 of IgG4 PE) and 370bp (IL4.Y124D).

To obtain larger amounts of the IgG4 PE variant hinge-CH2-CH3 fragment and the IL4.Y124D fragment, the purified and digested PCR products are ligated into Bluescript KS+<sup>TM</sup> which is prepared by digestion with either XhoI and XbaI for the hinge-CH2-CH3 of IgG4 PE fragment or EcoRV and XhoI for the IL4.Y124D fragment, followed by GeneClean<sup>TM</sup>. A Bluescript KS+/hinge-CH2-CH3 of IgG4 PE recombinant and a Bluescript KS+/IL4.Y124D recombinant are thus generated. Large amounts of these DNAs are produced using the Promega "Magic Maxiprep" method. The IgG4 PE hinge-CH2-CH3 fragment is excised from the Bluescript recombinant using XhoI and XbaI. The resulting fragment of approximately 690bp is purified by GeneClean<sup>TM</sup> to generate large amounts of the IgG4 PE hinge-CH2-CH3 XhoI/XbaI fragment. The IL4.Y124D fragment is excised from the Bluescript recombinant using EcoRV and XhoI and the resulting fragment of approximately 370bp is purified by GeneClean<sup>TM</sup>.

A vector is prepared into which to ligate the hinge-CH2-CH3 of IgG4 PE and IL4.Y124D fragments by digesting pDB951 with EcoRV and XbaI as described for the derivation of pDB952.

The three fragments described (IL4.Y124D EcoRV/XhoI, hinge-CH2-CH3 of IgG4 PE variant XhoI/XbaI and the 5.8Kb fragment resulting from EcoRV/XbaI digestion of pDB951) are ligated together to form plasmid pDB953 using a DNA ligation kit from Amersham (product code RPN 1507), incubating the reactions at 16°C overnight. The ligation reaction products are transformed into Promega JM109 competent cells (high efficiency) and plated onto Luria Broth agar containing ampicillin at 50µg/ml. Transformants are cultured in Luria Broth (containing ampicillin at 50µg/ml) and DNA prepared using Promega "Magic Minipreps". Production of an IL4.Y124D/IgG4 PE variant recombinant DNA is verified by restriction digests, and the complete IL4.Y124D and hinge-CH2-CH3 IgG4 PE variant regions are verified by DNA sequencing. Table 9 describes the sequence of the coding region only of the IL4.Y124D/IgG4 PE fusion molecule, and Table 10 contains the amino acid sequence of the fusion protein. The IL4.Y124D/IgG4 PE recombinant DNA is prepared and purified using caesium chloride gradients and the DNA used to transiently transfect HeLa cells.

## 2. Expression of the fusion protein

5 HeLa cells were grown in MEM $\alpha$  medium (Gibco) with 10% foetal calf serum and 1% glutamine. For the assay,  $1 \times 10^6$  HeLa cells were seeded in 15mls RPMI-1640 medium with 10% newborn calf serum, 1% glutamine ("seeding medium"), in a 75cm<sup>2</sup> flask, four days prior to transfection. On the day prior to transfection, a further 12.5mls seeding medium was added to each flask. On the day of transfection, the medium was changed to 15mls of "transfection medium" (MEM  
10 medium with Earle's salts containing 10% newborn calf serum and 1% non essential amino acids), at time zero. At time +3 hours, 25 $\mu$ g of the appropriate DNA in 0.125M CaCl<sub>2</sub>, 1x HBS (HEPES buffered saline) was added to the cells. At time +7 hours, the cells were subjected to a glycerol shock (15%v/v) and then left to incubate  
15 overnight in 12.5mls seeding medium containing 5mM sodium butyrate. The next day the cells were washed with PBS (Dulbecco's phosphate buffered saline) and 12.5mls "harvest medium" (RPMI-1640 with 2% of a 7.5% stock sodium bicarbonate solution) was added. After a further 24 hour incubation, the supernatants were removed, centrifuged at 1000rpm for 5 minutes to remove cell debris and stored at  
20 either 4°C or -20°C.

## 3. Biological Activity

For assay of supernatant for IL4 antagonist activity: using the method described in Spits et al., J. Immunology 139, 1142 (1987), human peripheral blood  
25 lymphocytes were incubated for three days with phytohaemagglutinin, a T cell mitogen, to upregulate the IL4 receptor. The resultant blast cells were then stimulated for a further three days with IL4. Proliferation was measured by the incorporation of <sup>3</sup>H thymidine.

The IL4.Y124D/IgG4 PE chimera inhibited <sup>3</sup>H thymidine incorporation by  
30 human peripheral blood T lymphocytes stimulated with 133pM IL4 in a dose dependent manner.

## Example 4. Mammalian Expression vector containing DNA coding for IL4.Y124D/IgG4 PE.

35

### 1. Construction of DNA

The pCDN vector (Aiyar, N., Baker, E., Wu, H-L., Nambi, P., Edwards, R.M., Trill, J.J., Ellis, C., Bergsma, D. Molecular and Cellular Biochemistry 131:75-86, 1994) contains the CMV promoter, a polylinker cloning region, and the BGH polyadenylation

region. This vector also contains a bacterial neomycin phosphotransferase gene (NEO) inserted between the  $\beta$ -globin promoter and SV40 polyadenylation region for Geneticin<sup>TM</sup> selection, the DHFR selection cassette inserted between the  $\beta$ -globin promoter and BGH polyadenylation region for methotrexate (MTX) amplification, an ampicillin resistance gene for growth in bacteria, and a SV40 origin of replication.

To insert the IL4.Y124D/IgG4 PE cDNA, the pCDN vector was prepared by digesting with NdeI and BstXI as follows: 15 $\mu$ g of DNA was incubated with 30 units of BstXI in react 2 (Gibco-BRL) at 55°C for 1 hour, and ethanol precipitated. The resulting DNA was digested with NdeI in react 2 at 37°C for 1 hour, and ethanol precipitated. The IL4.Y124D/IgG4 PE fragment was prepared from pDB953 (Example 3.1) by digesting with BstXI and NdeI as follows: 15 $\mu$ g of DNA was incubated with 30 units of BstXI in react 2 at 55°C for 1 hour, and ethanol precipitated. The resulting DNA was digested with NdeI in react 2 at 37°C for 1 hour, and ethanol precipitated.

The IL4.Y124D/IgG4 PE NdeI/BstXI and pCDN NdeI/BstXI fragments were ligated together to form the plasmid pCDN-IL4.Y124D/IgG4 PE. The ligation was achieved using 2 units of T4 DNA Ligase (Gibco BRL) with T4 DNA Ligase buffer. The reactions were incubated at 16°C overnight. The ligation reaction products were transformed into Gibco-BRL DH5a competent cells (subcloning efficiency) and plated onto Luria Broth agar containing 75  $\mu$ g/ml ampicillin. Transformants were cultured in Luria Broth (containing ampicillin at 50  $\mu$ g/ml) and DNA prepared by alkaline lysis. Production of a pCDN-IL4.Y124D/IgG4 PE DNA was confirmed by restriction digests. The complete sequence of the recombinant IL4.Y124D/IgG4 PE DNA was confirmed by sequencing. The pCDN-IL4.Y124D/IgG4 PE recombinant DNA was prepared and purified using Qiagen columns and the DNA was used to transiently infect COS cells and electroporated into CHO cells to create stable clones.

## 2. Expression of the Fusion Protein

### a) Transient Expression in COS

COS-1 cells were grown in DMEM medium with 10% fetal bovine serum. For the transfection, cells were seeded at  $2 \times 10^5$  cells into a 35mm tissue culture dish 24 hours prior. A solution containing 1 $\mu$ g of DNA in 100 $\mu$ l of DMEM without serum is added to a solution containing 6 $\mu$ l of LIPOFECTAMINE Reagent (Gibco-BRL) in 100 $\mu$ l of DMEM without serum, gently swirled and incubated at room temperature for 45 minutes. The cells are washed once with serum free DMEM. 0.8ml of serum free DMEM is added to the DNA-LIPOFECTAMINE SOLUTION, mixed gently and the diluted solution is overlayed on the cells. The cells are incubated at 37°C for 5 hours, then 1ml of DMEM containing 20% fetal bovine serum is added. The cells are assayed 48-72 hours later to determine expression levels.

**b) Electroporation into CHO cells**

- 5 CHO cells, ACC-098 (a suspension cell line derived from CHO DG-44, Urlaub, G., Kas, E., Carothers, A.M. and Chasin, L.A. Cell, 33. 405-412, 1983) were grown in serum free growth medium WO 92/05246. 15µg of the pCDN-IL4.Y124D/IgG4 PE  
10 plasmid was digested using 30 units of NotI at 37°C for 3 hours to linearize the plasmid, and precipitated with ethanol. The resulting DNA was resuspended in 50ul of 1 X TE (10mM Tris, pH 8.0, 1mM EDTA). The DNA was electroporated into 1 X  
10<sup>7</sup> ACC-098 cells, using a Bio Rad Gene Pulser set at 380V and 25µF. The cells  
were resuspended into growth medium at 2.5 X 10<sup>4</sup> cells/ml, and 200µl of the cell  
suspension was plated into each well of a 96 well plate. 48 hours later the medium  
was switched to growth medium containing 400µg/ml G418 (Geneticin). Twenty one  
15 days post selection, conditioned medium from the colonies which arose were screened by Elisa assay. The highest expressing colonies were transferred to 24 well plates in order to be scaled up.

Table 1. DNA sequence of COSFcLink vector, 6367bp

SEQ ID No:1	
	60
5	GACGTCGACGGATCGGGAGATCGGGGATCGATCCGTCGACGTACGACTAGTTATTAATAG
	120
	TAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTT
	180
	ACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATGACGTCAATAATG
	240
	ACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGACTAT
	300
	TTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCT
	360
	ATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCCAGTACATGACCTTATGG
10	420
	GACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGG
	480
	TTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTACGGGGATTTCGAAGTCTC
	540
	CACCCCATGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCAAAA
	600
	TGTCGTAACAACCTCCGCCCCATTGACGCAAAATGGGCGGTAGGCGTGTACGGTGGGAGGTC
	660
	TATATAAGCAGAGCTGGGTACGTGAACCGTCAGATCGCCTGGAGACGCCATCGAATTCCG
15	720
	TTACCTGCAGATATCAAGCTAATTCGGTACCGAGCCCAAATCGGCCGACAAAACCTCACAC
	780
	ATGCCACCGTGCCCGACCTGAACCTCCTGGGGGACCGTCAGTCTTCTCTTCCCCC
	840
	AAAACCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGA
	900
	CGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA
	960
	TAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGT
20	1020
	CCTCACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAA
	1080
	CAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGA
	1140
	ACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTACGCT
	1200
	GACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGG
	1260
	GCAGCCGAGAACAACCTACAAGACCACGCTCCCGTGCTGGACTCCGACGGCTCCTTCTT
25	1320
	CCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG
	1380
	CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCC
	1440
	GGGTAAATGAGTGATGTAGTCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCA
	1500
	GCCATCTGTTGTTTGCCCCCTCCCCCGTGCCTTCCCTTGACCCTGGAAGGTGCCACTCCCAC
	1560
	TGTCCTTTCTTAATAAAATGAGGAAATGCGATTGCTGTGAGTAGGTGTCATTCTAT
30	1620
	TCTGGGGGGTGGGGTGGGGCAGGACAGCAAAGGGGGAGGATTGGGAAGACAATAGCAGGCA
	1680
	TGCTGGGGATGCGGTGGGCTCTATGGAACCACTGAGGGCTCGAGGGGGGATCTCCCGATC
	1740
	CCCAGCTTTGCTTCTCAATTTCTTATTTGCATAATGAGAAAAAAGGAAATTAATTTTA
	1800
	ACACCAATTACGTAGTTGATTGAGCAATGCGTTGCCAAAAGGATGCTTTAGAGACAGT
	1860
	GTTCTCTGCACAGATAAGGACAAACATTATTAGAGGGAGTACCCAGAGCTGAGACTCCT
35	1920
	AAGCCAGTGAGTGGCACAGCATTCTAGGGAGAAATATGCTTGTATCACCAGGCCTGAT
	1980
	TCCGTAGAGCCACACCTTGGTAAGGGCCAATCTGCTCACACAGGATAGAGAGGGCAGGAG
	2040
	CCAGGGCAGAGCATATAAGGTGAGGTAGGATCAGTTGCTCCTCACATTGCTTCTGACAT
	2100
	AGTTGTGTTGGGAGCTTGGATAGCTTGGACAGCTCAGGGCTGCGATTTCGCGCCAACTT
	2160
	GACGGCAATCCTAGCGTGAAGGCTGGTAGGATTTTATCCCCGCTGCCATCATGGTTCGAC
40	2220
	CATTGAACTGCATCGTCGCGGTGTCCCAAAATATGGGGATTGGCAAGAACGGAGACCTAC
	2280
	CCTGGCCTCCGCTCAGGAACGAGTTCAAGTACTTCCAAAGAATGACCACAACCTCTTCAG
	2340
	TGGAAGGTAAACAGAATCTGGTGATTATGGGTAGGAAAACCTGGTTCTCATTCTGAGA
	2400
	AGAATCGACCTTTAAAGGACAGAATTAATATAGTTCTCAGTAGAGAACTCAAAGAACCAC
	2460
	CACGAGGAGCTCATTTTCTTGCCAAAAGTTTGGATGATGCCTTAAGACTTATTGAACAAC
45	2520
	CGGAATTGGCAAGTAAAGTAGACATGGTTTGGATAGTCGGAGGCAGTTCTGTTTACCAGG
	2580
	AAGCCATGAATCAACCAGGCCACCTTAGACTCTTGTGACAAGGATCATGCAGGAATTTG
	2640
	AAAGTGACACGTTTTTCCAGAAATTGATTTGGGGAAATATAAACTTCTCCAGAATACC
	2700
	CAGGCGTCTCTCTGAGGTCCAGGAGGAAAAAGGCATCAAGTATAAGTTGAAGTCTACG
	2760
	AGAAGAAAGACTAACAGGAAGATGCTTTCAAGTTCTCTGCTCCCTCCTAAAGCTATGCA
50	2820
	TTTTTATAAGACCATGCTAGCTTGAAGTGTGTTTATTGCAGCTTATAATGGTTACAAATAA
	2880
	AGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTTCACTGCATTCTAGTTGTGGT
	2940
	TTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCAACGATAGCTTATCTGTGGGC
	3000
	GATGCCAAGCACCTGGATGCTGTTGGTTTCCTGCTACTGATTTAGAAGCCATTGCCCCC

	TGAGTGGGGCTTGGGAGCACTAACTTTCTCTTTCAAAGGAAGCAATGCAGAAAGAAAAGC	3060
	ATACAAAGTATAAGCTGCCATGTAATAATGGAAGAAGATAAGGTTGTATGAATTAGATTT	3120
	ACATACTTCTGAATTGAACTAAACACCTTTAAATTCTTAAATATATAACACATTTTCATA	3180
	TGAAAGTATTTTACATAAGTAACCTCAGATACATAGAAAACAAAGCTAATGATAGGTGTCC	3240
5	CTAAAAGTTCATTTATTAATTCTACAAATGATGAGCTGGCCATCAAAATTCAGCTCAAT	3300
	TCTTCAACGAATTAGAAAGAGCAATCTGCAAACTCATCTGGAATAACAAAAAACCTAGGA	3360
	TAGCAAAACTCTTCTCAAGGATAAAAGAACCTCTGGTGGAATCACCATGCCTGACCTAA	3420
	AGCTGTACTACAGAGCAATTGTGATAAAACTGCATGGTACTGATATAGAAACGGACAAG	3480
	TAGACCAATGGAATAGAACCCACACACCTATGGTCACTTGATCTTCAACAAGAGAGCTAA	3540
10	AACCATCCACTGGAAAAAAGACAGCATTTTCAACAAATGGTGTGGCACAACCTGGTGGTT	3600
	ATCATGGAGAAGAATGTGAATTGATCCATTCCAATCTCCTTGTTACTAAGGTCAAATCTAA	3660
	GTGGATCAAGGAACTCCACATAAAACCAGAGACACTGAACTTATAGAGGAGAAAGTGGG	3720
	GAAAAGCCTCGAAGATATGGGCACAGGGGAAAAATTCCTGAATAGAACAGCAATGGCTTG	3780
	TGCTGTAAGATCGAGAATTGACAAATGGGACCTCATGAAACTCCAAAGCTATCGGATCAA	3840
15	TTCTCCAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGCGGCTCGG	3900
	CCTCTGCATAAATAAAAAAATTAGTCAGCCATGCATGGGGCGGAGAATGGGCGGAACTG	3960
	GGCGGAGTTAGGGGCGGGATGGGCGGAGTTAGGGCGGGACTATGGTTGCTGACTAATTG	4020
	AGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCTGGTT	4080
	GCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTT	4140
20	CCACACCTTAAGTACACACATTCCACAGAATTAATCCCGATCCCGTCGACCTCGAGAG	4200
	CTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAATTCC	4260
	ACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTA	4320
	ACTCACATTAATTGCGTTGCGCTCACTGCCCCGCTTCCAGTCGGGAAACCTGTCGTGCCA	4380
	GCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCCTATTGGGCGCTCTTC	4440
25	CGCTTCTCGCTCACTGACTCGCTGCGCTCGGTTCGGCTGCGGCGAGCGGTATCAGC	4500
	TCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACAT	4560
	GTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCTTGCTGGCGTTTTT	4620
	CCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCG	4680
	AAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTC	4740
30	TCCTGTTCCGACCCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGT	4800
	GGCGCTTTTCTCAATGCTCAGCTGTAGGTATCTCAGTTCCGTGTAGGTGCTTCGCTCAA	4860
	GCTGGGCTGTGTGCACGAACCCCGTTACGCCGCTGCGCCTTATCCGGTAACCTA	4920
	TCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAA	4980
	CAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCTAA	5040
35	CTACGGGTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTT	5100
	CGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTT	5160
	TTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGAT	5220
	CTTTTCTACGGGCTCTGACGCTCAGTGAACGAAACTCACGTTAAGGGATTTTGGTCAT	5280
	GAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTAAATC	5340
40	AATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGC	5400
	ACCTATCTCAGCGATCTGTCTATTTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGA	5460
	GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGCGAGA	5520
	CCCACGCTCACC GGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCG	5580
	CAGAAGTGGTCTGCAACTTTATCCGCCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGC	5640
45	TAGAGTAAGTAGTTCCGCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCAT	5700
	CGTGGTGTACGCTCGTCTGTTTGGTATGGCTTCATTACAGCTCCGGTCCCAACGATCAAG	5760
	GCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCTCCGAT	5820
	CGTTGTGAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAA	5880
	TTCTCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAA	5940
50	GTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGCGTCAATACGGGA	6000
	TAATACCGCGCCACATAGCAGAACTTTAAAGTGCTCATCATTGGAAAACGTTCTTCGGG	6060
	GCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTTCGATGTAACCCACTCGTGC	6120
	ACCCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGG	6180
	AAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACT	6240



CTTCCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACAT 6300  
 ATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTCCCCGAAAAAGT 6360  
 GCCACCT 6367

5

Table 2. DNA sequence of encoded Y124D-IgG1 fusion molecule in COSFcLink vector, 6926bp

SEQ ID No:2

10	GACGTCGACGGATCGGGAGATCGGGGATCGATCCGTCGACGTACGACTAGTTATTAATAG	60
	TAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTT	120
	ACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATGACGTCAATAATG	180
	ACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTAT	240
	TTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCT	300
15	ATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGG	360
	GACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGG	420
	TTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTC	480
	CACCCCATGACGTCAATGGGAGTTTGTGTTTGGCACAAAATCAACGGGACTTTCCAAAA	540
	TGTCGTAACTCCGCCCCATTGACGCAATGGGCGGTAGGCGGTACGGTGGGAGGTC	600
20	TATATAAGCAGAGCTGGGTACGTGAACCGTCAGATCGCCTGGAGACGCCATCGAATTCGG	660
	TTACCTGCAGATGGGCTGCAGGAATTCCGCATTGCAGAGATAATTGTATTTAAGTGCCTA	720
	GCTCGATACAATAAACGCCATTTGACCATTACCCACATTGGTGTGCACCTCCAAGCTTAC	780
	CTGCCATGGGTCTCACCTCCCACTGCTTCCCCCTCTGTTCTTCTGCTAGCATGTGCCG	840
	GCAACTTGTCCACGGACACAAGTGCATATCACCTTACAGGAGATCATCAAACTTTGA	900
25	ACAGCTCACAGAGCAGAAGACTCTGTGCACCGAGTTGACCGTAACAGACATCTTTGCTG	960
	CCTCCAAGAACACAACAGAGTGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCAGT	1020
	TCTACAGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGACTGCACAGCAGTTCCACA	1080
	GGCACAAAGCAGCTGATCCGATTCTGAAACGGCTCGACAGGAACCTCTGGGGCTGGCGG	1140
	GCTTGAATTCTGTCTGTGAAGGAAGCCAACAGAGTACGTTGGAAGTCTTGGAAA	1200
30	GGCTAAAGACGATCATGAGAGAGAAAGACTCAAAGTGTTGAGCGGTACCGAGCCCAAT	1260
	CGGCCGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGT	1320
	CAGTCTTCTCTTCCCCCCTAAACCAAGGACACCCTCATGATCTCCCGGACCCCTGAGG	1380
	TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACG	1440
	TGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA	1500
35	CGTACCGGTTGGTGCAGCGTCTCACCCTGCTGCACAGGACTGGCTGAATGGCAAGGAGT	1560
	ACAAGTGAAGGTCTCCAACAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAG	1620
	CCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGA	1680
	CCAAGAACCAGGTGAGCCTGACCTGCTGGTCAAAGGCTTCTATCCAGCGACATCGCCG	1740
	TGGAGTGGGAGAGCAATGGGCAGCCGAGAACAATAAGACCACGCCTCCCGTGCTGG	1800
40	ACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGC	1860
	AGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGA	1920
	AGAGCCTCTCCCTGTCTCCGGGTAAATGAGTGTAGTCTAGAGCTCGCTGATCAGCCTCGA	1980
	CTGTGCCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCTTGACCC	2040
	TGGAAGGTGCCACTCCCACTGTCTTTCTTAATAAAATGAGGAAATTGCATCGCATTGTC	2100
45	TGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATT	2160
	GGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAACCAGCTGGGGCTC	2220
	GAGGGGGGATCTCCCGATCCCAGCTTTGCTTCTCAATTTCTTATTTGCATAATGAGAAA	2280
	AAAAGGAAATTAATTTTAAACCAATTCAGTAGTTGATTGAGCAAATGCGTTGCCAAAA	2340
	AGGATGCTTTAGAGACAGTGTTCTCTGCACAGATAAGGACAAACATTATTCAGAGGGAGT	2400
50	ACCCAGAGCTGAGACTCCTAAGCCAGTGAGTGGCACAGCATTCTAGGGAGAAATATGCTT	2460
	GTCATCACCGAAGCCTGATTCCGTAGACCCACACCTTGGTAAGGGCCAATCTGCTCACAC	2520

	AGGATAGAGAGGGCAGGAGCCAGGGCAGAGCATATAAGGTGAGGTAGGATCAGTTGCTCC	2580
	TCACATTGCTTCTGACATAGTTGTGTTGGGAGCTTGGATAGCTTGGACAGCTCAGGGCT	2640
	GCGATTTTCGCGCCAACTTGACGGCAATCCTAGCGTGAAGGCTGGTAGGATTTTATCCCC	2700
	GCTGCCATCATGGTTCGACCATTGAAGTGCATCGTCGCCGTGTCCAAAATATGGGGATT	2760
5	GGCAAGAACGGAGACCTACCCTGGCCTCCGCTCAGGAACGAGTTCAAGTACTTCCAAAGA	2820
	ATGACCACAACCTCTTCAGTGAAGGTAACAGAATCTGGTGATTATGGGTAGGAAAACC	2880
	TGGTTCCTCATTCTGAGAAGAATCGACCTTTAAAGGACAGAATTAATATAGTTCTCAGT	2940
	AGAGAACTCAAAGAACCACCGAGGAGCTCATTTTCTTGCCAAAAGTTTGGATGATGCC	3000
	TTAAGACTTATTGAACAACCGGAATTGGCAAGTAAAGTAGACATGGTTTGGATAGTCGGA	3060
10	GGCAGTTCTGTTTACCAGGAAGCCATGAATCAACCAGGCCACCTTAGACTCTTTGTGACA	3120
	AGGATCATGCAGGAATTTGAAAGTGACACGTTTTTCCAGAAATTGATTGGGGAAATAT	3180
	AAACTTCTCCAGAAATACCCAGGCGTCTCTCTGAGGTCCAGGAGGAAAAAGCATCAAG	3240
	TATAAGTTTGAAGTCTACGAGAAGAAAGACTAACAGGAAGATGCTTTCAGTTCTCTGCT	3300
	CCCCTCCTAAAGCTATGCATTTTTATAAGACCATGCTAGCTTGAAGTTGTTATTGCAGC	3360
15	TTATAATGGTTACAAATAAAGCAATAGCATCACAAATTCACAAATAAAGCATTTTTTTC	3420
	ACTGCATTCTAGTTGTGGTTGTGCCAACTCATCAATGTATCTTATCATGTCTGGATCAA	3480
	CGATAGCTTATCTGTGGCGATGCCAAGCACCTGGATGCTGTTGGTTTCTCTGCTACTGAT	3540
	TTAGAAGCCATTTGCCCCCTGAGTGGGGCTTGGGAGCACTAAGTTTCTCTTCAAAGGAA	3600
	GCAATGCAGAAAGAAAAGCATACAAAGTATAAGCTGCCATGTAATAATGGAAGAAGATAA	3660
20	GGTTGTATGAATTAGATTACATACTTCTGAATTGAACTAAACACCTTTAAATCTTAA	3720
	ATATATAACACATTTTCATATGAAAGTATTTTACATAAGTAACTCAGATACATAGAAAACA	3780
	AAGCTAATGATAGGTGTCCCTAAAGTTTCAATTTATTAATTCTACAAATGATGAGCTGGCC	3840
	ATCAAAATTCAGCTCAATTCTTCAACGAATTAGAAAGAGCAATCTGCAAACTCATCTGG	3900
	AATAACAAAAAACCTAGGATAGCAAAACTCTTCTCAAGGATAAAAGAACCTCTGGTGGGA	3960
25	ATCACCATGCCTGACCTAAAGCTGTACTACAGAGCAATTGTGATAAAACTGCATGGTAC	4020
	TGATATAGAAACGGACAGTAGACCAATGGAATAGAACCCACACACCTATGGTCACTTGA	4080
	TCTTCAACAAGAGAGCTAAAACCTCCACTGGAAAAAGACAGCATTTTCAACAAATGGT	4140
	GCTGGCACAACTGGTGGTTATCATGGAGAAGATGTGAATTGATCCATTCCAATCTCCTT	4200
	GTACTAAGGTCAAATCTAAGTGGATCAAGGAATCCACATAAAACCAGAGACACTGAAAC	4260
30	TTATAGAGGAGAAAGTGGGGAAGCCTCGAAGATATGGGCACAGGGGAAAAATTCCTGA	4320
	ATAGAACAGCAATGGCTTGTGCTGTAAGATCGAGAATTGACAAATGGGACCTCATGAAAC	4380
	TCCAAAGCTATCGGATCAATTCCCTCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCA	4440
	GAGGCCGAGGCGGCTCGGCCTCTGCATAAATAAAAAAATTAGTCAGCCATGCATGGGG	4500
	CGGAGAATGGGCGGAACCTGGGCGGAGTTAGGGGCGGGATGGGCGGAGTTAGGGGCGGGAC	4560
35	TATGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGG	4620
	GGACTTTCCACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGC	4680
	TGGGGAGCCTGGGGACTTTCCACACCTTAAGTACACACATTCCACAGAATTAATTCCTG	4740
	ATCCCGTCGACCTCGAGAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAATT	4800
	GTTATCCGCTCACAATTCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGG	4860
40	GTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCCGCTTTCCAGT	4920
	CGGGAACCTGTGCTGCCAGCTGCATTAATGAATCGGCCAACCGCGGGGAGAGGCGGTT	4980
	TGCGTATTGGGCGCTCTTCCGCTTCCCTCACTGACTCGCTGCGCTCGGTGCTTCCGGC	5040
	TGCGGCGAGCGGTATCAGCTCACTCAAAGCGGTAATACGGTTATCCACAGAATCAGGGG	5100
	ATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGG	5160
45	CCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGAC	5220
	GCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATACCAGGCGTTTCCCCCTG	5280
	GAAGCTCCCTCGTGCCTCTCCTGTTCCGACCTGCGCTTACCGGATACCTGTCCGCCT	5340
	TTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCAGCTGTAGGTATCTCAGTTCCG	5400
	TGTAGTTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTTACGCCCCGACCGCT	5460
50	GCGCCTTATCCGGTAACTATCGTCTTGAGTCAACCCGGTAAGACACGACTTATCGCCAC	5520
	TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGT	5580
	TCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTC	5640
	TGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACA	5700
	CCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGAT	5760

	CTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAACCTCAC	5820
	GTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATT	5880
	AAAAATGAAGTTTAAATCAATCTAAAGTATATAGTAAACTTGGTCTGACAGTTACC	5940
	AATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTG	6000
5	CCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTG	6060
	CTGCAATGATACCGCGAGACCCACGCTCACC GGCTCCAGATTTATCAGCAATAAACCAGC	6120
	CAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCCTGCAACTTTATCCGCCTCCATCCAGTCTA	6180
	TTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTG	6240
	TTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTACAGCT	6300
10	CCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAGCGGTTA	6360
	GCTCCTTCGGTCTCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTTATCACTCATGG	6420
	TTATGGCAGCACTGCATAATTCTCTTACTGTCTATGCCATCCGTAAGATGCTTTTCTGTGA	6480
	CTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATCGGGCGACCGAGTTGCTCTT	6540
	GCCCCGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAGTGCTCATCA	6600
15	TTGGAACAGTCTTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTT	6660
	CGATGTAACCCACTCGTGCACCCAACCTGATCTTCAGCATCTTTTACTTTTACCAGCGTTT	6720
	CTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGA	6780
	AATGTTGAATACTCATACTCTTCCTTTTCAATATTATTGAAGCATTATCAGGGTTATT	6840
	GTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTTCCGC	6900
20	GCACATTTCCCGGAAAAGTGCCACCT	6926

Table 3. DNA sequence of IL4.Y124D/IgG1 fusion molecule coding region, 1164bp

25	SEQ ID No:3	
	ATGGGTCTCACCTCCCAACTGCTTCCCCCTCTGTTCTTCTCTGCTAGCATGTGCCGGCAAC	60
	TTTGTCCACGGACACAAGTGCGATATCACCTTACAGGAGATCATCAAACTTTGAACAGC	120
	CTCAGAGCAGAAAGACTCTGTGCACCGAGTTGACCGTAACAGACATCTTTGCTGCCTCC	180
	AAGAACACAACCTGAGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCAGTTCTAC	240
30	AGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGACTGCACAGCAGTTCCACAGGCAC	300
	AAGCAGCTGATCCGATTCTGAAACGGCTCGACAGGAACCTCTGGGGCCTGGCGGGCTTG	360
	AATTCCTGTCTGTGAAGGAAGCCAACCAGAGTACGTTGGAACCTTCTTGAAAGGCTA	420
	AAGACGATCATGAGAGAGAAAGACTCAAAGTGTTTCGAGCGGTACCGAGCCCAATCGGCC	480
	GACAAAACCTCACACATGCCACCGTGCCACGACCTGAACTCCTGGGGGGACCGTCAGTC	540
35	TTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACA	600
	TGCGTGGTGGTGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGAC	660
	GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGAGGAGCAGTACAACAGCACGTAC	720
	CGGGTGGTCAGCGTCTCACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAG	780
	TGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAA	840
40	GGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAG	900
	AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAG	960
	TGGGAGAGCAATGGGCAGCCGAGACAACACTACAAGACCACGCCTCCCGTGTGACTCC	1020
	GACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGG	1080
	AACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGC	1140
45	CTCTCCCTGTCTCCGGGTAAATGA	1164

Table 4. Sequence of encoded IL4.Y124D/IgG1 fusion protein, 387aa

50	SEQ ID No:4	
	1 MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIKTLNS LTEQKTLCTE	
	51 LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH	

	101	KQLIRFLKRL	DRNLWGLAGL	NSCPVKEANQ	STLENFLERL	KTIMREKDSK
	151	CSSGTEPKSA	DKTHTCPPCP	APELLGGPSV	FLFPPKPKDT	LMISRTPEVT
	201	CVVVDVSHED	PEVKFNWYVD	GVEVHNAKTK	PREEQYNSTY	RVVSVLTVLH
	251	QDWLNGKEYK	CKVSNKALPA	PIEKTISKAK	GQPREPQVYT	LPPSRDELTK
5	301	NQVSLTCLVK	GFYPSDIAVE	WESNGQPENN	YKTTTPVLDS	DGSFFLYSKL
	351	TVDKSRWQQG	NVFSCSVMHE	ALHNHYTQKS	LSLSPGK*	

Table 5. DNA sequence of synthetic IgG4 cDNA, 1006bp

10

## SEQ ID No:5

	GCTTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAG	60
	AGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTG	120
	TGGAACCTCAGGCGCCCTGACCAGCGCGTGCACACCTTCCCGCTGTCTACAGTCTCTCA	180
15	GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACC	240
	TACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCC	300
	AAATATGGTCCCCCATGCCCATCATGCCAGCACCTGAATTTCTGGGGGGACCATCAGTC	360
	TTCCTGTTCCCCCAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTACAG	420
	TGCGTGGTGGTGACGTGAGCCAGGAAGACCCGAGGTCCAGTTCAACTGGTACGTGGAT	480
20	GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC	540
	CGTGTGGTCAGCGTCTCACCCTCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAG	600
	TGCAAGGTCTCCAACAAAGGCCTCCCGTCATCGATCGAGAAAACCATCTCCAAAGCCAAA	660
	GGGCAGCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAG	720
	AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGGAG	780
25	TGGGAGAGCAATGGGCAGCCGAGAGAACTACAAGACCACGCCTCCCGTGCTGGACTCC	840
	GACGGATCCTTCTTCTCTACAGCAGGCTAACCCTGGACAAGAGCAGGTGGCAGGAGGGG	900
	AATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACAGAAGAGC	960
	CTCTCCCTGTCTCTGGGTAAATGAGTGTAGTCTAGATCTACGTATG	1006

30

Table 6. DNA sequence of IL4.Y124D/IgG4 fusion molecule coding region, 1149bp

## SEQ ID No:6

	ATGGGTCTCACCTCCCAACTGCTTCCCCCTGTGTTCTTCTGCTAGCATGTGCCGCAAC	60
35	TTTGTCCACGGACACAAGTGCGATATCACCTTACAGGAGATCATCAAACCTTTGAACAGC	120
	CTCACAGAGCAGAAGACTCTGTGCACCGAGTTGACCGTAACAGACATCTTTGTGCTCTCC	180
	AAGAACAACAAGTGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCAGTTCTAC	240
	AGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGACTGCACAGCAGTTCCACAGGCAC	300
	AAGCAGCTGATCCGATTCTTGAAACGGCTCGACAGGAACCTCTGGGGCCTGGCGGGCTTG	360
40	AATTCTGTCTGTGAAGGAAGCCAACCAGAGTACGTTGGAAAACCTTCTTGAAAGGCTA	420
	AAGACGATCATGAGAGAGAAAGACTCAAAGTGCTCGAGCGAGTCCAAATATGGTCCCCCA	480
	TGCCCATCATGCCCAGCACCTGAATTTCTGGGGGGACCATCAGTCTTCTGTTCCCCCA	540
	AAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTACGTTGCGTGGTGGTGGAC	600
	GTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCAT	660
45	AATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTTCAGCGTC	720
	CTCACCGTCTCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC	780
	AAAGGCCTCCCGTCATCGATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAG	840
	CCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTACGCTG	900
	ACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG	960
50	CAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGATCCTTCTTC	1020
	CTCTACAGCAGGCTAACCCTGGACAAGAGCAGGTGGCAGGAGGGAATGTCTTCTCATGC	1080
	TCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTG	1140
	GGTAAATGA	1149

Table 7. Sequence of encoded IL4.Y124D/IgG4 fusion protein, 382aa

5	SEQ ID No:7	
	1	MGLTSQLLPP LFFLLACAGN FVHGKCDIT LQEIIKTLNS LTEQKTLCTE
	51	LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH
	101	KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKDSK
	151	CSSESKYGPP CPSCPAPEFL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
10	201	VSQEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTYRVVSV LTVLHQDWLN
	251	GKEYKCKVSN KGLPSSIEKT ISKAKGQPRE PQVYTLPPSQ EEMTKNQVSL
	301	TCLVKGFYPS DIAVEWESNG QPENNYKTP PVLDSDGSFF LYSRLTVDKS
	351	RWQEGNVFSC SVMHEALHNH YTQKSLSLSL GK*

15

Table 8. DNA sequence of IgG4 PE variant, 984bp

	SEQ ID No:8	
	GCTAGTACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAG	60
20	AGCACgGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTG	120
	TGGAACCTCAGGCGCCCTGACCAGCGCGGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA	180
	GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACC	240
	TACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCC	300
	AAATATGGTCCCCCATGCCACcCATGCCAGCgCCTGAaTTtgaGGGGGGACCATCAGTC	360
25	TTCCTGTTCCCCCAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACG	420
	TGCGTGGTGGTGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGAT	480
	GGCGTGAGGTTGCATAATGCCAAGACAAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC	540
	CGTGTGGTCAGCGTCTCACCCTCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAG	600
	TGCAAGGTCTCCAACAAAGGCCTCCCGTCAcTgATCGAGAAAACCATCTCCAAGCCAAA	660
30	GGGCAGCCCCGAGAGCCACAGGTGTACACCTGCCCCCATCCAGGAGGAGATGACCAAG	720
	AACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGGAG	780
	TGGGAGAGCAATGGGCAGCCGAGAACAACTACAAGACCAGCCTCCCGTGTGGACTCC	840
	GACGGaTCCTTCTTCTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGG	900
	AATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGC	960
35	CTCTCCGTGTCTCTGGGTAAATGA	984

Table 9. DNA sequence of IL4.Y124D/IgG4 PE fusion molecule coding region, 1149bp

40	SEQ ID No:9	
	ATGGGTCTCACCTCCCAACTGCTTCCCCCTCTGTTCTTCTGCTAGCATGTGCCGGCAAC	60
	TTTGTCCACGGACACAAGTGCGATATCACCTTACAGGAGATCATCAAACTTTGAACAGC	120
	CTCACAGAGCAGAAGACTCTGTGCACCGAGTTGACCGTAACAGACATCTTTGCTGCCTCC	180
	AAGAACAACAAGTGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCAGTTCTAC	240
45	AGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGACTGCACAGCAGTTCCACAGGCAC	300
	AAGCAGCTGATCCGATTCTTCTGAAACGGCTCGACAGGAACCTCTGGGGCCTGGCGGGCTTG	360
	AATTCTGTCTGTGAAGGAAGCCAACCAAGTACGTTGGAAAACCTTCTTGGAAAGGCTA	420
	AAGACGATCATGAGAGAGAAAGACTCAAAGTGCTCGAGCGAGTCCAAATATGGTCCCCCA	480
	TGCCCACCATGCCCAGCgCCTGAATTTGAGGGGGGACCATCAGTCTTCTGTTCCCCCA	540
50	AAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTACGTGCGTGGTGGTGGAC	600
	GTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCAT	660
	AATGCCAAGACAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTC	720

CTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC 780  
 AAAGGCCTCCCGTCaTCgATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAG 840  
 CCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTGAGCCTG 900  
 ACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG 960  
 5 CAGCCGGAGAACAACCTACAAGACCAGCCTCCCGTGCTGGACTCCGACGGaTCCTTCTTC 1020  
 CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGC 1080  
 TCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTG 1140  
 GGTAATGA 1149

10

Table 10. Sequence of encoded IL4.Y124D/IgG4 PE variant fusion protein, 382aa

SEQ ID No:10

	1	MGLTSQLLPP	LFFLLACAGN	FVHGKCDIT	LQEIIKTINS	LTEQKTLCTE
15	51	LTVTDIFAAS	KNTTEKETFC	RAATVLRQFY	SHHEKDTRCL	GATAQQFHRH
	101	KQLIRFLKRL	DRNLWGLAGL	NSCPVKEANQ	STLENFLERL	KTIMREKDSK
	151	CSSESQYGGP	CPPCPAPEFE	GGPSVFLFPP	KPKDTLMISR	TPEVTCVVVD
	201	VSQEDPEVQF	NWYVDGVEVH	NAKTKPREEQ	FNSTYRVVSV	LTVLHQDWLN
	251	GKEYKCKVSN	KGLPSSIEKT	ISKAKGQPRE	PQVYTLPPSQ	EEMTKNQVSL
20	301	TCLVKGFYPS	DIAVEWESNG	QPENNYKTTT	PVLDSGGSFF	LYSRLTVDKS
	351	RWQEGNVFSC	SVMHEALHNH	YTQKSLSLSL	GK*	

**CLAIMS**

1. A soluble protein having IL4 and/or IL13 antagonist or partial antagonist activity,  
5 comprising an IL4 mutant or variant fused to least one human immunoglobulin constant domain or fragment thereof.
2. A compound according to claim 1, wherein at least one amino acid, naturally occurring  
10 in wild type IL4 at any one of positions 120 to 128 inclusive, is replaced by a different natural amino acid.
3. A compound according to claim 2, wherein the tyrosine naturally occurring at  
position 124 is replaced by a different natural amino acid.
- 15 4. A compound according to claim 3, wherein the tyrosine naturally occurring at position 124 is replaced by aspartic acid.
5. A compound according to any one of the preceding claims, wherein the  
20 immunoglobulin is of the IgG subclass
6. A compound according to claim 5, wherein the constant domain(s) or fragment thereof is the whole or a substantial part of the constant region of the heavy chain of human IgG.
- 25 7. A compound according to claim 5, wherein the constant domain(s) or fragment thereof is the whole or a substantial part of the constant region of the heavy chain of human IgG4.
8. A compound according to claim 1, having the amino acid sequence represented by  
30 SEQ ID No:4, SEQ ID No:7 or SEQ ID No:10.
9. A process for preparing a compound according to any one of the preceding claims, which process comprises expressing DNA encoding said compound in a recombinant host cell and recovering the product.

10. A process according to claim 9, which comprises:
- i) preparing a replicable expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes said compound;
  - ii) transforming a host cell with said vector;
  - 5 iii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said compound; and
  - iv) recovering said compound.
11. A DNA polymer comprising a nucleotide sequence that encodes a compound
- 10 according to any one of claims 1 to 8.
12. A DNA polymer according to claim 11, which comprises or consists of the sequence of SEQ ID No:3, SEQ ID No:6 or SEQ ID No:9.
13. A replicable expression vector comprising a DNA polymer according to claim 11.
14. A host cell transformed with a replicable expression vector according to claim 13.
- 15 A pharmaceutical composition comprising a compound according to any one of
- 20 claims 1 to 8 and a pharmaceutically acceptable carrier.
16. A method of treating conditions resulting from undesirable actions of IL4 and/or IL13 which comprises administering to the sufferer an effective amount of a compound according to claim 1.
- 25 17. A compound according to any one of claims 1 to 8, for use in therapy.
18. A compound according to any one of claims 1 to 8, for use in the treatment of conditions resulting from undesirable actions of IL4 and/or IL13.
- 30 19. Use of a compound according to any one of claims 1 to 8 in the manufacture of a medicament for use in the treatment of conditions resulting from undesirable actions of IL4 and/or IL13.



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 95/03036

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/62 C07K14/54 C07K16/00 C07K19/00 A61K38/19  
A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 464 533 (BEHRINGWERKE) 8 January 1992 cited in the application see claims; examples ---	1-7, 10, 11, 13-19
Y	WO,A,93 10235 (SEBALD) 27 May 1993 cited in the application see the whole document ---	1-7, 10, 11, 13-19
Y	EMBO JOURNAL, vol. 12, no. 7, July 1993 EYNHAM, OXFORD GB, pages 2663-2670, S.M. ZURAWSKI ET AL 'Receptors for Interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction ' see the whole document ---	1-7, 10, 11, 13-19

-/--

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search

12 December 1995

Date of mailing of the international search report

03.01.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+ 31-70) 340-3016

Authorized officer

Le Cornec, N

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 95/03036

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MOLECULAR IMMUNOLOGY, vol. 30, no. 1, January 1993 pages 105-108, S. ANGAL ET AL 'A single amino acid substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody' cited in the application see the whole document ---	1-7,10, 11,13-19
Y	WO,A,88 07089 (MEDICAL RESEARCH COUNCIL) 22 September 1988 see the whole document & EP,A,0 307 434 (MEDICAL RESEARCH COUNCIL) cited in the application ---	1-7,10, 11,13-19
A	EP,A,0 367 166 (TAKEDA CHEMICAL INDUSTRIES. LTD.) 9 May 1990 see claims -----	1

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 95/ 03036

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 16  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although this claim is directed to a method of treatment of the human/animal body (Rule 39.1(iv), the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 95/03036

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-464533	08-01-92	AU-B- 655421	22-12-94
		AU-B- 7935791	02-01-92
		CA-A- 2045869	29-12-91
		JP-A- 5247094	24-09-93
-----			
WO-A-9310235	27-05-93	DE-A- 4137333	19-05-93
		AU-A- 2928292	15-06-93
		CA-A- 2123315	27-05-93
		CZ-A- 9401185	15-12-94
		EP-A- 0613499	07-09-94
		HU-A- 66826	30-01-95
		JP-T- 7501522	16-02-95
		NO-A- 941681	06-05-94
-----			
WO-A-8807089	22-09-88	AU-B- 600575	16-08-90
		AU-B- 1480388	10-10-88
		DE-D- 3883899	14-10-93
		DE-T- 3883899	31-03-94
		EP-A, B 0307434	22-03-89
		EP-A- 0351410	24-01-90
		WO-A- 8807054	22-09-88
		GB-A, B 2209757	24-05-89
		JP-T- 1502875	05-10-89
-----			
EP-A-367166	09-05-90	AU-B- 622724	16-04-92
		AU-B- 4391089	03-05-90
		JP-A- 2209898	21-08-90
-----			